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Ubiquitous upstream repression sequences control activation of the inducible arginase gene in yeast

(*Saccharomyces cerevisiae*/repressor binding site/transcriptional activation/transcriptional induction)

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ABSTRACT **Expression of the yeast arginase gene (*CAR1*) responds to both induction and nitrogen catabolite repression. Regulation is mediated through sequences that both positively and negatively modulate *CAR1* transcription. A short sequence, 5′-TAGCCGCCGAGGG-3′, possessing characteristics of a repressor binding site, plays a central role in the induction process. A fragment containing this upstream repression sequence (URS*1*) repressed gene expression when placed either 5′ or 3′ to the upstream activation sequences of the heterologous gene *CYC1*. Action of the URS and its cognate repressor was overcome by *CAR1* induction when the URS was situated cis to the *CAR1* flanking sequences. This was not observed, however, when it was situated downstream of a heterologous *CYC1* upstream activation sequence indicating that URS function is specifically neutralized by cis-acting elements associated with *CAR1* induction. Searches of sequences in various gene banks revealed that URS*1*-like sequences occur ubiquitously in genetic regulatory regions including those of bacteriophage λ, yeast, mammalian, and viral genes. In a significant number of cases the sequence is contained in a region associated with negative control of yeast gene regulation. These data suggest the URS identified in this work is a generic repressor target site that apparently has been conserved during the evolution of transcriptional regulatory systems.**

Identification of sequences and proteins that mediate control of eukaryotic gene expression is central to our understanding of this process. Recent work has shown that sequences responsible for gene activation and control, upstream activation sequences (UAS) as they are called, tend to be short (1, 2). For yeast genes responding to regulation by the general amino acid control system, for example, a 6-base-pair (bp) sequence appears to be sufficient for activation and control (3). This conclusion is based on the observation that placement of this short sequence 5′ to the TATA box of a heterologous gene lacking its UAS sequence supported gene expression (4). Moreover, expression responded to amino acid starvation just as it does for *HIS4* and a variety of other genes encoding amino acid biosynthetic enzymes (4). Recently, Hope and Struhl have shown that the *GCN4* gene product binds to this sequence (5), an observation they interpreted to indicate that this protein was responsible for activation of the genes in this regulon (set of similarly regulated genes). A similar motif of regulation is being found for the *GAL, PHO, SUC, DAL, CAR*, and mating type responsive genes, to name just a few.

The number of yeast genes responding to well-characterized negative control systems is considerably smaller. The most notable representatives are those associated with mating type (6), *CYC7* (7), enolase (8), the genes encoding arginase (*CAR1*) and ornithine aminotransferase (*CAR2*) (9), and *CTT1* (10). The *CAR1* and *CAR2* genes are the focus of this work. Expression of these genes is induced by arginine and is subject to nitrogen catabolite repression when cells are provided with a readily used nitrogen source such as asparagine (11).

Deletion analysis of the 5′ flanking region of the *CAR1* gene revealed an UAS with characteristics similar to those mentioned above (refs. 12–14 and unpublished work). In addition to this positively acting UAS site, a negatively acting site, or upstream repression sequence (URS*1*), was tentatively identified by the existence of a cis-dominant, constitutive mutation (*CAR1-0*$^-$ allele) shown to be a C-to-G transversion at position −153 relative to the start of *CAR1* translation (9). The *CAR1-0*$^-$ mutation resulted in constitutive expression of the normally inducible *CAR1* gene (11). The objective of the work reported here was to determine directly whether or not the C-to-G transversion at position −153 was mutationally inactivating a repressor binding site, as we suggested earlier, and to identify the function of this sequence and the regulatory protein associated with it. Preliminary reports of this work have appeared earlier (15, *).

MATERIALS AND METHODS

Yeast strain RH218 was used throughout this work as the transformation recipient. Yeast transformations and culture conditions have appeared (11). Cell breakage and β-galactosidase assays were done by the method of Guarente (16); units are those used by Miller (17).

Synthesis of oligonucleotides was performed on an Applied Biosystems (Foster City, CA) model 380B DNA synthesizer at the University of Tennessee, Memphis Molecular Resource Center. Oligonucleotides were purified by gel electrophoresis. Vectors used here were derived from plasmids pLG312 and p1G669Z (18, 19) (Fig. 1).

To construct plasmids pRS218–220, synthetic oligonucleotides were ligated into the *Sal* I site of plasmid pRS53. Plasmid pRS91 was prepared by linearizing plasmid pRS45 (9) with *Xma* III. The DNA was then treated with S1 nuclease, phenol extracted, ethanol precipitated, and religated with T4 DNA ligase. Deletion endpoints were identified by Maxam–Gilbert sequence analysis (20). Plasmid pRS179 was derived from plasmid pRS154 by replacement of the 2-μm autonomously replicating sequence (ARS) with the *TRP1*/ARS*1* fragment (21). The inserts of plasmids pRS185 and pRS186 were *Bgl* II–*Bam*HI fragments derived from plasmids pRS82 and pRS115, respectively. pRS82 is a plasmid carrying an upstream deletion of all wild-type *CAR1*

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Abbreviations: UAS, upstream activation sequence; URS, upstream repression sequence; ARS, autonomously replicating sequence; UIS, upstream induction sequence.

*Sumrada, R. A. & Cooper, T. G., 13th International Conference on Yeast Genetics and Molecular Biology, Aug. 31–Sept. 5, 1986, Banff, AB, Canada, p. 105 (abstr.).

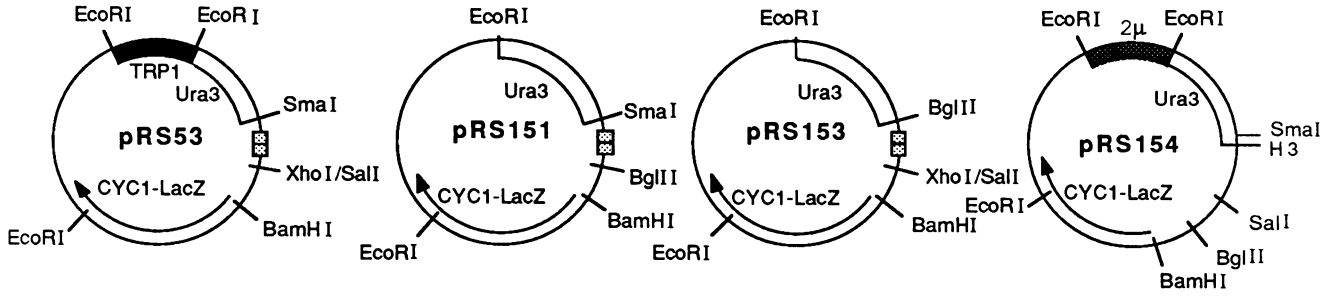

FIG. 1. Vectors used in this work.

DNA 5′ of position −185. pRS115 is a similar plasmid carrying a deletion of *CAR1-0*$^-$ DNA upstream of position −175 (positions are relative to the ATG of the coding sequence; unpublished work). These inserts were isolated and ligated into plasmid pRS151 digested with *Bgl* II; the *TRP1*/ARS*1* *Eco*RI fragment (21) was then ligated into the construction. Plasmid pRS210 was similarly derived except that plasmid pRS153 was used as vector.

RESULTS

Deletion of the Upstream Repression Sequence. Past work from our laboratory demonstrated that a single C-to-G transversion at position −153 (*CAR1-0*$^-$ mutant), of the *CAR1* gene, resulted in constitutive expression (9). This phenotype might be accounted for in one of two ways. The transversion mutation may have generated a new UAS that is not subject to arginine system control (induction and repression). Alternatively, it may have inactivated the binding site of a negatively acting repressor protein that regulates the *CAR1* induction system. The distinguishing characteristic of these alternatives is their response to deletion. In the case of a mutationally generated UAS, deletion of the pertinent sequences would result in loss of constitutive expression. In contrast, deletion of a repressor binding site would be expected to produce the same phenotype as its inactivation by a point mutation. Therefore, we constructed a 13-bp deletion spanning position −153 of *CAR1*. The size of the deletion was decided on the basis that position −153 of *CAR1* was situated in a 13-bp region with exact homology to a sequence similarly positioned in *CAR2*, a gene regulated in the same way as *CAR1* (ref. 9 and unpublished work). An RH218 transformant containing the 13-bp deletion plasmid (pRS91) was grown in various media along with transformants containing wild-type (pRS46) or *CAR1-0*$^-$ (pRS45) *LACZ* fusion plasmids as controls. As shown in Fig. 2, the wild-type *CAR1–LACZ* fusion plasmid (pRS46) supported low level β-galactosidase production when grown with glutamate as nitrogen source (an uninduced condition). Arginase (*LACZ* expression) was induced about 10-fold when arginine replaced glutamate in the medium (an induced condition). Expression was sensitive to nitrogen catabolite repression as indicated by the low level of β-galactosidase activity observed when asparagine was provided along with arginine as a nitrogen source. In contrast to this normal physiological profile, the *CAR1-0*$^-$*–LACZ* fusion plasmid (pRS45) supported constitutive β-galactosidase synthesis in the absence of the inducer arginine. Moreover, enzyme production was largely resistant to nitrogen catabolite repression. The deletion plasmid (pRS91) exhibited a physiological profile of gene expression identical to that of the *CAR1-0*$^-$*–LACZ* fusion plasmid pRS45. These data are more consistent with the argument that the point mutation in the *CAR1-0*$^-$ strain inactivated a repressor binding site than they are with the explanation that an aberrant upstream activation sequence had been generated by the C-to-G transversion. It is also important to note that plasmid pRS91 supported the same level of β-galactosidase production as the wild-type plasmid (pRS46) assayed in fully induced cells. Full level induction in wild-type cells yields the same levels of enzyme activity as cells in which the putative repressor binding site has been deleted. This observation leads to the conclusion that induction is epistatic to repression.

Repression of a Heterologous Gene by URS*1*. We directly tested the ability of sequences in the −153 region to repress gene expression by placing them either upstream or downstream of the heterologous *CYC1* UAS. This was done by cloning an 89-bp *Bgl* II–*Bam*HI fragment derived from a wild-type or *CAR1-0*$^-$ mutant plasmid (plasmids pRS82 and pRS115, respectively) (9) into either the *Sma* I or *Xho* I sites that flank the *CYC1* UAS (Fig. 3). The resulting plasmids were transformed into a wild-type yeast strain (RH218), and transformants were assayed for β-galactosidase activity. The unmodified *CYC1–LACZ* fusion plasmid (pRS53) supported production of 104 units of activity (Fig. 3). Placement of a wild-type *Bgl* II–*Bam*HI fragment, containing position −153 (plasmid pRS185), downstream of the *CYC1* UAS resulted in a 13-fold decrease in *LACZ* expression (levels were comparable to those observed after deletion of the *CYC1* UAS, as shown for plasmid pRS179). Similar repression of *LACZ* expression was observed when the sequence was placed 5′ to the *CYC1* UAS (plasmid pRS210). Note that repression of the

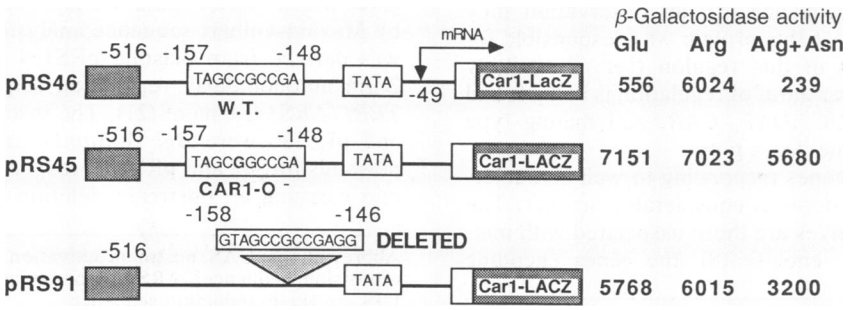

FIG. 2. Mutational inactivation of a negatively acting site in the upstream region of *CAR1*. Plasmids pRS46, pRS45, and pRS91 were transformed into yeast strain RH218. Transformants were grown on glutamate, arginine, or arginine plus asparagine as nitrogen source.

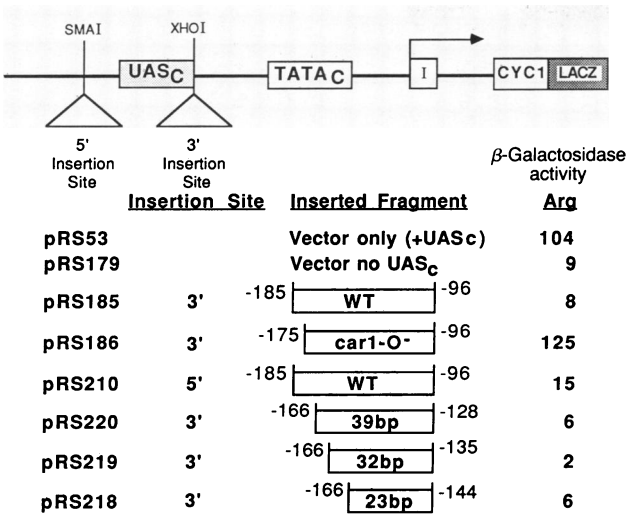

FIG. 3. Repression of the *CYC1* UAS by wild-type and mutant fragments and synthetic oligonucleotides derived from the upstream region of *CAR1*. Plasmids pRS53, pRS185, pRS186, and pRS210 contained DNA derived from deletion plasmids of the *CAR1* upstream region (unpublished work). Plasmids pRS218–220 contained synthetic oligonucleotides. All plasmids were transformed into strain RH218. The transformants were grown and assayed as in Fig. 2. Numbers above the fragment illustrations refer to coordinates in *CAR1*.

CYC1 UAS occurred under induced conditions for *CAR1* gene expression. Repression of *CYC1* UAS function could not be overcome by growth conditions that induce *CAR1* expression—in sharp contrast to the result seen in Fig. 2, where induction of *CAR1* did overcome the effects of repression.

Because insertion of the 89-bp fragment between the *CYC1* UAS and the TATA box could theoretically be detrimental to expression of the gene, the experiment was repeated using a nearly identical *CAR1-0*$^-$ fragment (−96 to −175) in place of the wild type. A quite different result was observed; not only was *CYC1–LACZ* expression undiminished by insertion of the 79-bp mutant fragment downstream of the *CYC1* UAS (plasmid pRS186), but it was slightly increased (Fig. 3). Hence, repression of *CYC1–LACZ* expression by the wild-type −96 to −175 fragment was legitimate, and not due to a problem of altered spacing.

Localization of the URS. DNA fragments used in the above experiments were rather large, thereby precluding precise localization of the URS they contained. Therefore, we synthesized a number of double-stranded oligonucleotides corresponding to portions of the fragment used in the initial experiments. These fragments were cloned into the *Xho* I site downstream of the *CYC1* UAS and tested for their ability to repress gene expression. As shown in Fig. 3, the vector alone was able to support about 104 units of β-galactosidase activity when the *CYC1* UAS was present (plasmid pRS53) and about 9 units in its absence (plasmid pRS179). The latter value established the background level of plasmid-mediated gene expression. As observed earlier, the 89-bp wild-type *CAR1* fragment (plasmid pRS185) repressed expression to this background level. Successively shorter fragments of 39 bp (plasmid pRS220), 32 bp (plasmid pRS219), and 23 bp (plasmid pRS218) yielded similarly repressed levels. This suggests that a maximum of only 23 bp was sufficient for repression. The 13-bp homology that exists between the *CAR1* and *CAR2* genes is wholly contained within the fragment (9).

Homology Between the *CAR1* URS and Similar Sites in Other Genes. Localization of URS*1* to a 13- to 23-bp sequence afforded an opportunity to determine whether similar sequences were present in other genes whose structures had been reported. The analysis was conducted both visually from published sequences and by computer scanning of 8745 gene sequences (5.93 megabases) in the GenBank data base.† In general, we reported only homologies of >70% located in 5′ and other potentially interesting regulatory regions. Sixteen other yeast sequences were found to exhibit substantial homology with the 13-bp sequence found in the *CAR1* and *CAR2* genes (Table 1). A consensus sequence, 5′-TAGCCGCCGRRRR-3′ (where R = an unspecified purine nucleoside), was generated from these data. The extent of homology for the entire 13 bp ranged from 56 to 100%. However, for the symmetrical core sequence CCGCC 75 to 100% homology was observed with only conservative substitutions occurring

†National Institutes of Health (1986) Genetic Sequence Databank: GenBank (Research Systems Div., Bolt, Beranek, and Newman, Cambridge, MA), Tape Release 40.0.

Table 1. Homology between URS*1* and sequences upstream of various yeast genes

Gene	Position	Sequence													
CAR1	−160	C	G	G	T	A	G	C	C	G	C	C	G	A	G	G
CAR2	−180	g	G	c	T	A	G	C	C	G	C	C	G	A	c	G
G3PDH	−584	C	t	t	T	A	G	C	C	G	c	g	G	A	G	G
ENO1	−218	t	c	c	T	A	G	C	C	a	C	C	t	c	A	A
PYK1	−864	a	a	a	T	A	G	C	C	G	C	C	a	t	G	A
GAL–UAS$_g$	−233	t	a	G	a	A	G	C	C	G	C	C	G	A	G	c
CYC1	−241	a	G	a	g	A	t	C	C	G	C	C	A	G	t	G
CYC7	−288	a	G	t	c	c	c	C	C	G	C	C	G	A	G	G
CTA1	−247	a	a	t	T	A	G	C	C	G	C	g	c	A	A	G
CTT1	−236	C	t	c	T	g	G	C	t	G	C	a	G	G	c	t
HEM1	−380	C	t	G	c	g	G	C	C	G	C	g	G	G	c	G
MES1	−248	t	G	t	T	A	G	C	C	G	C	C	G	A	A	A
ILV2	−484	g	c	c	T	A	G	C	C	G	C	C	G	G	A	G
CYB2	−169	C	a	a	g	A	a	C	C	G	C	C	A	A	G	A
ARG4	−217	g	a	G	T	A	a	C	C	a	C	C	A	c	A	A
TOP1	−451	C	t	c	T	A	G	C	C	G	C	C	G	A	c	G
CS1	−349	C	c	G	c	A	G	C	C	G	C	C	c	G	G	A
MFα2	−188	a	c	a	g	A	G	t	C	G	C	C	G	t	G	G
					T	A	G	C	C	G	C	C	G	R	R	R
Consensus					11	15	14	17	17	16	18	14	11	14	13	16
Occurrence					18	18	18	18	18	18	18	18	18	18	18	18

R, purine.

Table 2. Homology between URS*1* and sequences upstream of various other genes

Gene	Position				Sequence										
URS*1* consensus					T	A	G		C	C	G	C	C	G	R R R R
ϕX174 protein n′ recognition	2333	t	t	t	T	t	G		C	C	G	C	t	G	A G G G
HSV origin	2376	g	c	g	g	g	G		C	C	G	C	C	G	G G t A
SV40 origin	96	c	c	t	a	a	t		C	C	G	C	C	c	c c a t
Human U1 snRNA	−305	c	c	a	g	A	G		C	C	G	C	C	A	A G G G
Chicken U1 snRNA	−130	c	g	t	g	A	G		C	C	G	C	C	G	t G C G
Sea urchin repetitive sequence	85	t	c	c	a	A	G		C	C	G	C	C	G	A A A A
BLV LTR	604	a	a	a	a	t	G		C	C	G	C	C	G	A G G G
Adeno E2 promoter		g	t	g	T	g	G		C	C	G	C	T	G	G A G A
λ repressor binding site			t	a	T	c	a		C	C	G	C	C	A	G A G G
SP1 binding site									C	C	G	C	C	C	

HSV, herpes simplex virus; SV40, simian virus 40; snRNA, small nuclear RNA; BLV, bovine leukemia virus; LTR, long terminal repeat.

in the nonhomologous positions. Sequences flanking the core exhibited somewhat less homology. However, in nearly every case the two base pairs on the 5′ side were purines as were the four bases on the 3′ side. In each of the yeast genes containing URS*1*, it is situated between the UAS of the gene and its TATA box. If a position bias exists, it is in favor of URS*1* being closer to the TATA box than is the UAS.

In a substantial number of cases the sequence with homology to URS*1* has been experimentally deleted. In each case where the experiment has been done [*CAR1* (this work), *ENO1* (8), *CYC1* (18), *CYC7* (22), and *CTT1* (23), deletion of the sequence resulted in a dramatic increase in gene expression (a 10- to 20-fold increase was common).

The URS*1* sequence also appeared in several eukaryotic sequences that potentially play regulatory roles (Table 2). Among them are the origins of replication for several mammalian cell viruses. Again it was the symmetrical core sequence CCGCC that possessed greatest homology. An additional interesting location for the core of the URS*1* was the core of the λ operator, which is known to participate in binding a repressor protein (24). The sequence CCGCC has been recently reported to be repeated six times in the SV40 early promoter, where it has been shown to be protected in footprint experiments (25). Those observations were interpreted to identify the sequence as the SP1 transcription factor binding site. In that case, CCGCC was associated with a positively acting element, thus raising the possibility that the sequence represents an efficient site for protein–DNA interaction. By this interpretation, whether the site is associated with a positive or negative action is a result of protein domains other than those associated with binding. This idea is not new. It has been substantiated by experiments with the *LEX* protein binding site and *LEX–GAL4* hybrid proteins (26).

DISCUSSION

We have demonstrated that the sequence 5′-TAGCCGC-CGAGGG-3′ functions as an URS when present adjacent to the UAS of yeast genes. While repression of gene transcription was observed whether URS*1* was placed 5′ or 3′ to an UAS, the observed effects were strongest in the 3′ position. The sequence plays a major role in regulation of the inducible *CAR1* gene as shown by the fact that constitutive *CAR1* expression was observed when the sequence was inactivated by point mutation or deletion. We have not identified how this sequence brings about repression, though it is likely that repression is mediated through the binding of a repressor protein to URS*1*. Other work has demonstrated that *CAR1* gene expression depends upon the operation of an UAS situated between positions −190 and −365 (refs. 12–14 and unpublished work). URS*1* is situated some 40 bp 3′ to those sequences and 11 bp 5′ of the TATA sequence.

One of the most important mechanistic questions about URS*1* operation and its physiological function concerns how putative repressor binding to URS*1* is rendered inoperative when *CAR1* expression is induced with arginine. Our data argue that inactivation must involve cis-acting regulatory elements. This conclusion derives from the location-specific response of URS*1* to induction. URS*1* function was inactivated by induction when located in its normal position 3′ to the UAS of *CAR1* (Fig. 2)—i.e., β-galactosidase activity observed in an induced wild-type fusion was the same as that found in the URS deletion. Induction-mediated inactivation of URS repression did not occur when the element was placed 3′ to the UAS of the heterologous gene, *CYC1* (Fig. 3). URS*1* could be inactivated when adjacent to the *CAR1* regulatory sequences and their cognate regulatory proteins but was incapable of *CAR1*-specific inactivation when located adjacent to other regulatory sites, such as those of the *CYC1* gene. This suggests that proteins interacting either with the *CAR1* UAS or more likely another site in the *CAR1* 5′ flanking region interact with URS*1* or proteins binding to it. We have designated this site the upstream induction sequence, or UIS (Fig. 4).

We suggest that induction of *CAR1* gene expression is accomplished as shown in Fig. 4. According to this model, transcription is activated by proteins that bind to the UAS. A protein binding to URS1, which is situated 3′ to the UAS, represses its operation. Induction is then brought about by a protein whose production or function is dependent upon inducer. This protein probably binds to the UIS and thereby inhibits operation of the putative repressor that binds to the URS. Inhibition of this repressor's function then leaves the transcriptional activator free to mediate transcription. From this reasoning it follows that the inducer inactivates transcriptional repression. URS*1* represses *CYC1* UAS operation but is refractile to inactivation by arginine-mediated *CAR1* induction because the target sequences required for the URS*1*-inactivating protein, the UIS, are not situated in the flanking region of the *CYC1* gene.

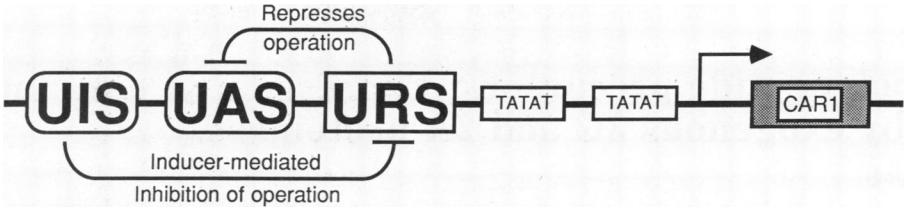

FIG. 4. A working model for the organization and operation of regulatory elements in the 5′ flanking region of the *CAR1* gene.

Elements of the above hypothesis may operate in the induction of human β interferon gene expression (27). That negatively acting sites occur in the regulatory regions of inducible eukaryotic genes from yeast to mammalian cells suggests that this motif is characteristic of eukaryotic induction systems *per se*. If so, a generic structure–function organization of regulatory regions similar to that proposed for *CAR1* will be found as more inducible, eukaryotic genes are studied. The homology studies reported here and noted earlier by Ruis and co-workers (10) and the deletion results from workers studying many of the genes containing the generic URS*1* forecast such findings.

An extension of the interpretation from the homology analysis is that the sequence identified as URS*1* may be a generic repressor binding sequence. This argument implies that the binding site itself does not contain the physiological specificity of repression but rather contains only the site for binding a generic repressor protein. In this model the physiological response of repressor protein binding would be controlled by another element and associated sites, each of which are gene specific. This interpretation of the data fits well with the above suggestion that inactivation of URS*1* is probably accomplished through cis-acting sites and proteins that are regulon specific. That the URS*1* core sequence CCGCC occurs in a wide variety of regulatory regions—ranging from the repressor binding site of bacteriophage λ to potentially interesting regulatory sequences in the animal viruses, SV40, and adenovirus—similarly points to URS*1* core sequence as a generic DNA–protein interaction site.

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